MANUSCRIPT TITLE: The effects of a single whole body cryotherapy exposure on physiological, performance and perceptual responses of professional academy soccer players following repeated sprint exercise

RUNNING TITLE: Cryotherapy and recovery from soccer

M. Russell [a], J. Birch [b], T. Love [b], C. J. Cook [c], R. M. Bracken [b], T. Taylor [d], E. Swift [d], E. Cockburn [e], C. Finn [b], D. Cunningham [b], L. Wilson [e], L. P. Kilduff [b]

[a] Health and Life Sciences, Northumbria University, Newcastle-upon-Tyne, United Kingdom

[b] Applied Sports Technology Exercise and Medicine Research Centre (A-STEM), Swansea University, Swansea, United Kingdom

[c] School of Sport, Health and Exercise Sciences, Bangor University, Bangor, United Kingdom

[d] West Ham United Football Club, London, United Kingdom

[e] London Sport Institute, Middlesex University, London, United Kingdom

Corresponding author: Professor Liam Kilduff
l.kilduff@swansea.ac.uk

Abstract word count:	241 words
Manuscript word count:	3214 words
Figures:	1
Tables:	1

MANUSCRIPT TITLE: The effects of a single whole body cryotherapy exposure on physiological, performance and perceptual responses of professional academy soccer players following repeated sprint exercise

RUNNING TITLE: Cryotherapy and recovery from soccer

ABSTRACT

In professional youth soccer players, the physiological, performance and perceptual effects of a single whole body cryotherapy (WBC) session performed shortly after repeated sprint exercise were investigated. In a randomized, counter-balanced and crossover design, 14 habituated English Premier League academy soccer players performed 15 x 30 m sprints (each followed by a 10 m forced deceleration) on two occasions. Within 20 min of exercise cessation, players entered a WBC chamber (Cryo: 30 s at -60°C, 120 s at -135°C) or remained seated (Con) indoors in temperate conditions (~25°C). Blood and saliva samples, peak power output (countermovement jump) and perceptual indices of recovery and soreness were assessed pre-exercise and immediately, 2 h and 24 h post-exercise. When compared to Con, a greater testosterone response was observed at 2 h (+32.5 ± 32.3 pg·ml^{-1}, +21%) and 24 h (+50.4 ± 48.9 pg·ml^{-1}, +28%) post-exercise (both P=0.002) in Cryo (trial x treatment interaction: P=0.001). No between trial differences were observed for other salivary (cortisol and testosterone/cortisol ratio), blood (lactate and Creatine Kinase), performance (peak power output) or perceptual (recovery or soreness) markers (all trial x treatment interactions: P>0.05); all of which were influenced by exercise (time effects: all P<0.05). A single session of WBC performed within 20 min of repeated sprint exercise elevated testosterone concentrations for 24 h but did not affect any other performance, physiological or perceptual measurements taken. While unclear, WBC may be efficacious for professional soccer players during congested fixture periods.

KEYWORDS: Creatine Kinase, fatigue, football, muscle damage, recovery

INTRODUCTION

Up to 120 h are required to restore disturbances in metabolic and physical performance markers following soccer match-play (19). We recently reported reduced countermovement jump (CMJ) performance and elevated Creatine Kinase (CK) concentrations in the 48 h after professional soccer matches of 90 min (21) and 120 min (23) durations. However, professional European soccer teams may play in excess of 60 competitive matches per season (6, 10) and thus at specific times of the year, multiple matches will be played within a single week (10). Although unclear (6) injury risk has been observed to increase when less than 96 h separates games (10) and the reduced recovery time between matches played in FIFA World Cup competitions is perceived by physicians to be a primary cause of injury in professional soccer players (18). Therefore, the ability to facilitate post-match recovery is desirable.

A number of interventions have been proposed to facilitate post-exercise recovery (19), including: nutritional strategies, cold water immersion, active recovery, compression garments, massage and electrical stimulation. An additional method is whole body cryotherapy (WBC), which typically involves exposure to very cold and dry air (-110 to -195°C) for a period of two to three minutes in a temperature-controlled chamber (2, 12, 14). As summarised in a narrative review (2), the therapeutic effects of repeated WBC exposures have been proposed to relate to changes in haematology (i.e., reduced haemolysis), muscular enzyme activity (i.e., reductions in circulating CK and lactate dehydrogenase concentrations) and modified hormonal responses (i.e., stimulated noradrenaline release). The importance of anti-oxidant capacity, inflammation, immunity and cardiac markers (2) and performance and perceptual indices of recovery have also been highlighted in WBC research (3).

The majority of studies employing WBC for recovery purposes have implemented multiple cold exposures; either, within a single day or throughout the week(s) following muscle damaging exercise. In elite Italian rugby players engaged in regular training, Banfi et al. (1) observed reductions relative to baseline values in muscle enzyme concentrations following five once-daily sessions of WBC over the course of a week. Similarly, numerous WBC exposures (3 min at -140 to -195°C) over a six day period improved the recovery of peak torque, rate of torque development, squat jump start power, and reduced muscle soreness at various time-points following damaging hamstring exercise (12). While multiple WBC sessions administered over the course of a 6 or 7 day period appears advantageous, the feasibility of such practices (i.e., repeated cold exposures) may be limited in soccer players who are competing in congested fixture schedules and thus likely have limited time (i.e., <96 h) between consecutive matches, and may also have travel commitments associated with away games.

Despite the use of WBC in athletic populations, limited studies have profiled the responses to an isolated bout of WBC performed after muscle damaging exercise. Of those that have, authors have typically examined the short term (i.e., ≤30 minutes) effects of cold exposure (25, 28). Furthermore, as training status (via habituation to eccentric contractions) has been proposed to modulate the efficacy of WBC (14), there is a need to determine the effects of a single WBC session in professional athletes. In a study examining the optimal duration of cryotherapy exposure, Selfe et al. (25) recently observed no differences in inflammatory markers between trials of one, two or three minutes performed on the day after a competitive Rugby League match. However, in the absence of a non-cryotherapy trial to determine the efficacy of the intervention *per se*, the effects of an isolated bout of WBC in professional athletes recovering from intermittent exercise remains to be determined. Therefore, the aim of this study was to examine the physiological, performance and perceptual effects (over 24 h) of a single bout of WBC performed shortly after repeated sprint exercise in professional soccer players.

METHODS

Experimental Approach to the Problem

To investigate the effects of a single WBC exposure performed after repeated sprint exercise on physiological, performance and perceptual responses, 14 professional academy soccer players were required to attend the testing venue on six occasions throughout a 14 day period. The first two of these sessions were preliminary visits that included procedural habituation whereas both main trials each required a further two separate visits.

Subjects

Following ethical approval from the Swansea University Ethics Committee, 14 male academy soccer players recruited from an English Premier League club (age: 18 ± 2 years, mass: 74.5 ± 5.5 kg, stature: 1.78 ± 0.05 m) provided written informed consent (and parental consent where players <18 years) before study involvement.

Procedures

Two main trials (Cryo: Whole body cryotherapy, Con: Control), separated by seven days, were completed in a randomized, counter-balanced and cross-over design. Main trials were performed in an enclosed sports hall that housed a 3G surface and was maintained at a temperature of ~25°C. To minimize the effects of circadian variation, the timing of measurements were consistent between trials. A light tactical training session, abstention from caffeine and replication of dietary intake was required in the 24 h before the first visit of each trial.

Upon arrival, resting capillary blood and saliva samples were taken before perceived muscle soreness and recovery was assessed. Following a short warm-up (~5 min), players performed two CMJ attempts (separated by 30 s) on a portable force platform (Type 92866AA, Kistler, Germany). A standardized 10 min warm-up (consisting of channel drills, dynamic stretches and progressive intensity sprinting) and 5 min passive rest then preceded 15 x 30 m timed (Brower timing system, Salt Lake City, Utah, USA) sprints that were each separated by 60 s rest (16). Each sprint required deceleration to a standstill within a 10 m zone, which contributes to the muscle damaging properties of the protocol (16). The protocol elicits similar distances covered at high intensity to those observed in a similar age group of professional players during match-play (22). Blood and saliva samples, perceived muscle soreness and recovery and CMJ performance were assessed immediately, 2 h and 24 h following the repeated sprint protocol and these measurements took ~10 min to complete on each occasion.

After providing blood and saliva samples and having completed the perceived recovery and soreness scales and CMJ testing, players commenced the WBC treatment in a purpose built temperature-controlled portable cryotherapy unit (BOC Cryotherapy Chamber, Linde, Surrey, UK) within 20 min of completing the repeated sprint protocol. Before entering the liquid nitrogen cooled chamber, players towel-dried themselves (to remove sweat) and wore minimal clothing (wearing shorts, socks, clogs, mask, gloves and a hat covering the auricles to avoid frostbite; 28); processes which were completed within 10 min. Players entered the first pre-cooling chamber (-60°C) for 30 s before moving into the second chamber (-135°C) for a further 120 s; a duration considered optimal when using a chamber of -135°C (25). Minimal deviations from the target temperature were observed when players moved between the pre-cooling and main chambers. Players were instructed to gently move fingers and legs to avoid tension, and to take slow, shallow breaths while in the chamber (28, 30). Upon leaving the chamber, players dressed in enough training attire to attenuate subjective feelings of cold and remained seated for ~95 min in the same room as used in the Con trial. In Con, players remained seated in a temperate environment (~25°C) for ~110 min. All players remained seated until the 2 h post-exercise assessments before being provided with a meal from a standardized menu and then leaving the laboratory. Players were requested to replicate their post-visit dietary intake between trials and no structured training was scheduled in the time between the 2 h and 24 h measurements. Verbal questioning of players on arrival for the 24 h post-exercise assessment supported adherence to these requests.

Peak power output was determined according to previously described methods (20, 29). Briefly, the instantaneous velocity and displacement of the player's center of gravity was derived from the vertical component of the ground reaction force (GRF) elicited during the CMJ and the participants' body mass. Instantaneous power output was determined using Equation 1 and the highest value produced from the two attempts performed at each time-point was deemed the peak power output.

Eq'n 1: Power (W) = vertical GRF (N) x Vertical velocity of centre of gravity ($m \cdot s^{-1}$)

Whole blood (5 μL), sampled from the fingertip (after immersion in warm water necessary for one participant during the Con trial), was analysed for lactate concentrations (Lactate Pro, Akray, Japan). A further 120 μL of blood (Microvette CB300 EDTA, Sarstedt AG & Co, Germany) was centrifuged at 3000 revolutions$\cdot min^{-1}$ for 10 min (Labofuge 400R, Kendro Laboratories, Germany) and plasma samples were stored at -70°C before subsequently being analysed for CK (Cobas Mira; ABX Diagnostics, Northampton, UK) concentrations. Samples were measured in duplicate (3% coefficient of variation) and recorded as a mean. Saliva samples were collected into sterile vials (LabServe, New Zealand) via passive drool (~2 ml over 2 min) which were then stored at -80°C. To minimize sample dilution, players were instructed to avoid eating, drinking warm fluids, and brushing of teeth in the two hours preceding sampling. Samples were analysed in duplicate using commercially available enzyme immunoassay kits (Salimetrics LLC, State College, PA, USA). The lowest detection limits for testosterone and cortisol were 0.001 $nmol \cdot L^{-1}$ and 0.08 $nmol \cdot L^{-1}$, respectively and inter-assay CV values were <10% in both cases. To eliminate inter-assay variance, samples for each player were analysed within the same assay kit (8). The perception of recovery was assessed using a 10-point likert scale (17) whereas a 7-point likert scale evaluated lower limb muscle soreness (27).

Statistical Analyses

Statistical analyses were carried out using SPSS Statistics software (IBM Inc., USA) with significance set at $P \leq 0.05$. Data are reported as mean ± standard deviation (SD). Paired samples t-tests were performed for between-trial comparisons of data expressed over a single time-point within a trial (i.e., mean and total sprint times). For data expressed over multiple time-points within a trial (i.e., individual sprint times, power output, blood lactate and Creatine Kinase concentrations, salivary testosterone and cortisol concentrations; including testosterone/cortisol ratio, and perceived soreness and recovery), between trial comparisons were investigated using two-way repeated measures analysis of variance (ANOVA; within-participant factors: trial x time). Where significant interaction effects were observed, trial was deemed to have influenced responses and simple main effect analyses were performed. Timing effects represent the main effect of time from the two-way repeated measures ANOVA analysis performed. Partial eta-squared (η^2) values were calculated and Bonferroni corrected *post-hoc* tests (with 95% Confidence Intervals; CI) were performed to isolate significant differences.

RESULTS

A two-way repeated measures ANOVA analysis revealed that individual sprint times were similar between trials (time x trial interaction: $F_{(6,78)}$=0.354 , P=0.905, η^2=0.026) and did not differ throughout the duration of the 15 x 30 m timed sprints (time effect: $F_{(3,44)}$=0.574, P=0.658, η^2=0.042). Paired samples t-tests highlighted that mean (Con: 4.34 ± 0.17 s, Cryo: 4.37 ± 0.23 s, P=0.572) and total (Con: 65.08 ± 2.56 s, Cryo: 65.56 ± 3.38 s, P=0.572) sprint times were comparable between trials.

Peak power output was not influenced by trial (time x trial interaction: $F_{(3,39)}$=0.762, P=0.522, η^2=0.055) but did differ according to timing (time effect: $F_{(3,39)}$=10.091, P<0.001, η^2=0.437). Peak power output reduced immediately post-exercise (P<0.001) by 134 ± 100 W (-3.2 ± 2.3%) but subsequently returned to pre-exercise values at 2 h (P=0.052) and 24 h (P>0.99) post-exercise (Table 1).

***** INSERT TABLE 1 NEAR HERE *****

Blood lactate concentrations were similar between trials (time x trial interaction: $F_{(2,21)}$=1.023, P=0.361, η^2=0.073, Table 1) but were influenced by timing (time effect: $F_{(1,16)}$=50.609, P<0.001, η^2=0.796). A 2.18 ± 1.01 mmol·L^{-1} increase from baseline values occurred immediately post-exercise (P<0.001) but blood lactate concentrations returned to pre-exercise values thereafter (P>0.05).

Concentrations of CK did not differ according to trial (time x trial interaction: $F_{(2,26)}$=0.733, P=0.491, η^2=0.053) but did vary due to timing of sample (time effect: $F_{(1,14)}$=243.872, P<0.001, η^2=0.949). Compared to pre-exercise values, CK was elevated by 14 ± 13%, 28 ± 10% and 253 ± 89% immediately (P=0.006), 2 h (P<0.001) and 24 h (P<0.001) post-exercise, respectively (Table 1).

Salivary testosterone concentrations were influenced by trial (trial x treatment interaction: $F_{(3,39)}$=6.231, P=0.001, η^2=0.326) and time of sample (time effect: $F_{(3,39)}$=6.275, P=0.001, η^2=0.326). Despite salivary testosterone being similar between trials at pre-exercise and immediately post-exercise (both P>0.05), Cryo elicited a greater salivary testosterone response at 2 h (+32.5 ± 32.3 $pg \cdot ml^{-1}$, +21 ± 21%) and 24 h (+50.4 ± 48.9 $pg \cdot ml^{-1}$, +28 ± 34%) post-exercise (both P=0.002) compared to Con (Figure 1).

***** INSERT FIGURE 1 NEAR HERE *****

Salivary cortisol concentrations did not differ according to trial (time x trial interaction: $F_{(3,39)}$=0.253, P=0.859, η^2=0.019) but did vary due to sampling time (time effect: $F_{(3,39)}$=13.998, P<0.001, η^2=0.518). Immediately post-exercise, salivary cortisol was similar to pre-exercise values (P=0.052) whereas significant reductions were observed at 2 h post-exercise (p=0.003). These reductions had dissipated at 24 h post-exercise (Figure 1). Salivary testosterone/cortisol ratios did not differ due to trial (time x trial interaction: $F_{(3,39)}$=0.696, P=0.560, η^2=0.051) but timing did influence the response (time effect: $F_{(2,28)}$=8.66, P=0.001, η^2=0.518). Post hoc analyses were unable to isolate these differences relative to pre-exercise values.

Perceived soreness (time x trial interaction: $F_{(3,39)}$=0.700, P=0.558, η^2=0.051) and recovery (time x trial interaction: $F_{(2,22)}$=0.245, P=0.752, η^2=0.019) were not influenced by trial but timing effects were significant ($F_{(3,39)}$=13.010, P<0.001, η^2=0.500, $F_{(3,39)}$=27.094, P<0.001, η^2=0.676, respectively). Significant changes were only observed immediately post-exercise (both P<0.001).

DISCUSSION

This study aimed to examine the physiological, performance, and perceptual effects of a single bout of WBC administered shortly after repeated sprint exercise in professional soccer players. Based on circulating CK concentrations yielded from capillary blood samples, our findings indicate that perturbations in selected physiological responses were not restored back to baseline values within a 24 h period. Moreover, a single WBC session increased testosterone concentrations at 2 h and 24 h post-exercise when compared to a Con trial despite no differences in CMJ performance, blood lactate and CK concentrations, and markers of perceived recovery. Although further investigation is warranted, these findings highlight a potential role for a single WBC exposure in the early stages of recovery from muscle damaging exercise in professional soccer players.

Contrary to previous authors (1, 31) Cryo did not influence blood CK concentrations when compared to Con (Table 1). Conversely, and despite torque loss being limited in the 48 h following trail running (14), Hausswirth et al. observed similar CK concentrations to that observed during a passive recovery trial after a single WBC exposure (14). Therefore, it has been proposed that repeated WBC sessions (a minimum of 5 to 10) are required before muscle membrane breakdown or exercise-induced cell permeability is modified to such an extent that the significant reductions in CK concentrations seen by previous authors (1, 31) become evident (14). Moreover, the elevated baseline CK concentrations of soccer players observed in this study and previously (21, 23, 26) may afford another explanation as to the lack of differences observed between trials in this variable and is likely attributable to residual levels of muscle damage still present from previous regular training (26).

Testosterone has been suggested to be a primary anabolic hormone involved in protein synthesis and protection against skeletal muscle degradation (15). Notwithstanding the debated role of endogenous hormones in the muscle hypertrophic and strength response (24), the 21% and 28% increases in testosterone at 2 h and 24 h post-exercise in Cryo versus Con, respectively, indicates a potentially favourable hormonal profile following a single exposure to WBC after soccer-specific exercise. Such findings corroborate observations of elevated testosterone concentrations following multiple WBC sessions (13) but are the first to be reported following a single bout of WBC that followed muscle damaging exercise in professional athletes. As testosterone concentrations influence training motivation (7), this finding may have important implications for practitioners during congested periods of competition.

The anti-inflammatory effects of WBC are a key factor purported to explain its efficacy (1, 2). As opposed to changes in lysosomal membrane stabilization which are apparent following multiple cryotherapy exposures (31), reductions in serum soluble intercellular adhesion molecule-1 (sICAM-1; mediator of the leukocyte response at the damaged tissue, resulting in a lower pro-inflammatory response, less reactive oxygen species and an increase in anti-inflammatory markers), have been proposed to explain the anti-inflammatory response to a single WBC session (11). Notably, low serum testosterone concentrations are significantly associated with elevated levels of inflammation (4). Speculatively, and given its role as a potential mediator of the inflammatory response in both healthy and clinical populations, the increases in testosterone observed at 2 h and 24 h post-exercise versus Con in this study may reflect reduced levels of inflammation following WBC. However, in the absence of inflammation data these proposed mechanisms should be interpreted with caution.

The increased testosterone concentrations observed against Con at 24 h post-exercise in Cryo may also reflect an increased sleep quality that has been reported previously (5). When compared to a previous night's sleep that did not follow a cryotherapy intervention, sleep quality was improved the night after WBC exposure (5). As sleep deprivation/restriction reduces testosterone concentrations (9), WBC may be beneficial for players experiencing disrupted sleeping patterns; perhaps resulting from travel and/or factors associated with evening kick-offs. Unfortunately, records of sleep quality were unavailable to support this supposition and warrants further investigation.

In contrast to previous studies that have implemented muscle damaging exercises that demonstrate low levels of ecological validity to soccer, such as; drop jumps combined with eccentric lower body exercise (12) and isokinetic unilateral knee extensor exercises (28), we used a repeated sprint protocol (16) that represents the high intensity distance covered in soccer match-play (22) and is also typical of some soccer training sessions. Although physiological measurements were not collected during exercise, players reported increased perceptions of soreness and a reduced recovery state immediately post-exercise (Table 1) while blood lactate concentrations reflected those observed following a soccer match and peak power output demonstrated a soccer-specific fatigue-related profile (21, 23). Furthermore, we observed increases in CK concentrations that were similar in magnitude to those reported following soccer match-play (21, 23). The reductions in cortisol concentrations observed 2 h post-exercise are likely explained by circadian rhythmicity given the non-significant effects of exercise on salivary cortisol when assessed immediately post-exercise and the subsequent restoration at 24 h. Therefore, our data highlights a potential role for WBC as a method of maintaining salivary testosterone concentrations in professional soccer players for up to 24 h following intense exercise.

PRACTICAL APPLICATIONS

A single session of WBC elicited greater testosterone concentrations for 24 h after repeated sprint exercise when compared to a passive recovery protocol despite selected physiological, performance and perceptual markers being unaffected. Although unclear, such findings may link to an attenuated inflammatory response to exercise, an enhanced sleep quality in the 24 h following cold exposure, and possibly have implications for subsequent training motivation. Consequently, WBC administered shortly after intermittent exercise may offer an ergogenic strategy for soccer players involved in a congested fixture or training period**.** A secondary finding of this study was that professional soccer players performing 15 x 30 m sprints (each followed by a forced deceleration within a 10 m zone) experienced a short term (up to 2 h) transient reduction in post-exercise muscle function (i.e., CMJ performance) and perturbations in circulating CK concentrations that required more than 24 h to return to baseline.

REFERENCES

1. Banfi G, Gianluca M, Alessandra B, Giada D, Gianvico M, Dugue B, and Corsi M. Effects of whole-body cryotherapy on serum mediators of inflammation and serum muscle enzymes in athletes. *J Therm Biol* 34: 55-59, 2009.
2. Banfi G, Lombardi G, Colombini A, and Melegati G. Whole-body cryotherapy in athletes. *Sports Med* 40: 509-517, 2010.
3. Bleakley CM, Bieuzen F, Davison GW, and Costello JT. Whole-body cryotherapy: empirical evidence and theoretical perspectives. *Open Access J Sports Med* 5: 25-36, 2014.
4. Bobjer J, Katrinaki M, Tsatsanis C, Lundberg Giwercman Y, and Giwercman A. Negative association between testosterone concentration and inflammatory markers in young men: a nested cross-sectional study. *PLoS One* 8: e61466, 2013.
5. Bouzigon R, Ravier G, Dugue B, and Grappe F. The use of whole-body cryostimulation to improve the quality of sleep in athletes during high level standard competitions. *Br J Sports Med* 48: 572, 2014.
6. Carling C, Le Gall F, and Dupont G. Are physical performance and injury risk in a professional soccer team in match-play affected over a prolonged period of fixture congestion? *Int J Sports Med* 33: 36-42, 2012.
7. Cook CJ, Crewther BT, and Kilduff L. Are free testosterone and cortisol concentrations associated with training motivation in elite athletes? *Psychol Sport Ex* 14: 882-885, 2013.
8. Crewther BT, Cook CJ, Gaviglio CM, Kilduff LP, and Drawer S. Baseline strength can influence the ability of salivary free testosterone to predict squat and sprinting performance. *J Strength Cond Res* 26: 261-268, 2012.
9. Dattilo M, Antunes HK, Medeiros A, Monico Neto M, Souza HS, Tufik S, and de Mello MT. Sleep and muscle recovery: endocrinological and molecular basis for a new and promising hypothesis. *Med Hypotheses* 77: 220-222, 2011.
10. Dupont G, Nedelec M, McCall A, McCormack D, Berthoin S, and Wisloff U. Effect of 2 soccer matches in a week on physical performance and injury rate. *Am J Sports Med* 38: 1752-1758.
11. Ferreira-Junior JB, Bottaro M, Loenneke JP, Vieira A, Vieira CA, and Bemben MG. Could whole-body cryotherapy (below -100 degrees C) improve muscle recovery from muscle damage? *Front Physiol* 5: 247, 2014.
12. Fonda B and Sarabon N. Effects of whole-body cryotherapy on recovery after hamstring damaging exercise: a crossover study. *Scand J Med Sci Sports* 23: e270-278, 2013.
13. Grasso D, Lanteri P, Di Bernardo C, Mauri C, Porcelli S, Colombini A, Zani V, Bonomi FG, Melegati G, Banfi G, and Lombardi G. Salivary steroid hormone response to whole-body cryotherapy in elite rugby players. *J Biol Regul Homeost Agents* 28: 291-300, 2014.
14. Hausswirth C, Louis J, Bieuzen F, Pournot H, Fournier J, Filliard JR, and Brisswalter J. Effects of whole-body cryotherapy vs. far-infrared vs. passive modalities on recovery from exercise-induced muscle damage in highly-trained runners. *PLoS One* 6: e27749, 2011.
15. Herbst KL and Bhasin S. Testosterone action on skeletal muscle. *Curr Opin Clin Nutr Metab Care* 7: 271-277, 2004.
16. Howatson G and Milak A. Exercise-induced muscle damage following a bout of sport specific repeated sprints. *J Strength Cond Res* 23: 2419-2424, 2009.
17. Laurent CM, Green JM, Bishop PA, Sjokvist J, Schumacker RE, Richardson MT, and Curtner-Smith M. A practical approach to monitoring recovery: development of a perceived recovery status scale. *J Strength Cond Res* 25: 620-628, 2011.
18. McCall A, Davison M, Andersen TE, Beasley I, Bizzini M, Dupont G, Duffield R, Carling C, and Dvorak J. Injury prevention strategies at the FIFA 2014 World Cup: perceptions and practices of the physicians from the 32 participating national teams. *Br J Sports Med* 49: 603-608.
19. Nedelec M, McCall A, Carling C, Legall F, Berthoin S, and Dupont G. Recovery in soccer: part I - post-match fatigue and time course of recovery. *Sports Med* 42: 997-1015, 2012.

20. Owen NJ, Watkins J, Kilduff LP, Bevan HR, and Bennett M. Development of a criterion method to determine peak mechanical power output in a countermovement jump. *J Strength Cond Res* 28: 1552-1558, 2014.
21. Russell M, Northeast J, Atkinson G, Shearer DA, Sparkes W, Cook CJ, and Kilduff L. The between-match variability of peak power output and Creatine Kinase responses to soccer match-play. *J Strength Cond Res*, 2015.
22. Russell M, Sparkes W, Northeast J, Cook CJ, Love TD, Bracken RM, and Kilduff LP. Changes in acceleration and deceleration capacity throughout professional soccer match-play. *J Strength Cond Res*, In press.
23. Russell M, Sparkes W, Northeast J, and Kilduff LP. Responses to a 120 minute reserve team soccer match: A case study focusing on the demands of extra-time. *J Sports Sci*, In press.
24. Schroeder ET, Villanueva M, West DD, and Phillips SM. Are acute post-resistance exercise increases in testosterone, growth hormone, and IGF-1 necessary to stimulate skeletal muscle anabolism and hypertrophy? *Med Sci Sports Exerc* 45: 2044-2051, 2013.
25. Selfe J, Alexander J, Costello JT, May K, Garratt N, Atkins S, Dillon S, Hurst H, Davison M, Przybyla D, Coley A, Bitcon M, Littler G, and Richards J. The effect of three different (-135 degrees C) whole body cryotherapy exposure durations on elite rugby league players. *PLoS One* 9: e86420, 2014.
26. Silva JR, Ascensao A, Marques F, Seabra A, Rebelo A, and Magalhaes J. Neuromuscular function, hormonal and redox status and muscle damage of professional soccer players after a high-level competitive match. *Eur J Appl Physiol* 113: 2193-2201, 2013.
27. Vickers AJ. Time course of muscle soreness following different types of exercise. *BMC Musculoskelet Disord* 2: 5, 2001.
28. Vieira A, Bottaro M, Ferreira-Junior JB, Vieira C, Cleto VA, Cadore EL, Simoes HG, Carmo JD, and Brown LE. Does whole-body cryotherapy improve vertical jump recovery following a high-intensity exercise bout? *Open Access J Sports Med* 6: 49-54.
29. West DJ, Owen NJ, Jones MR, Bracken RM, Cook CJ, Cunningham DJ, Shearer DA, Finn CV, Newton RU, Crewther BT, and Kilduff LP. Relationships between force-time characteristics of the isometric midthigh pull and dynamic performance in professional rugby league players. *J Strength Cond Res* 25: 3070-3075, 2011.
30. Wozniak A, Mila-Kierzenkowska C, Szpinda M, Chwalbinska-Moneta J, Augustynska B, and Jurecka A. Whole-body cryostimulation and oxidative stress in rowers: the preliminary results. *Arch Med Sci* 9: 303-308, 2013.
31. Wozniak A, Wozniak B, Drewa G, Mila-Kierzenkowska C, and Rakowski A. The effect of whole-body cryostimulation on lysosomal enzyme activity in kayakers during training. *Eur J Appl Physiol* 100: 137-142, 2007.

ACKNOWLEDGEMENTS

None to declare. No external financial support received. The results of the present study do not constitute endorsement by the NSCA.

FIGURE LEGEND

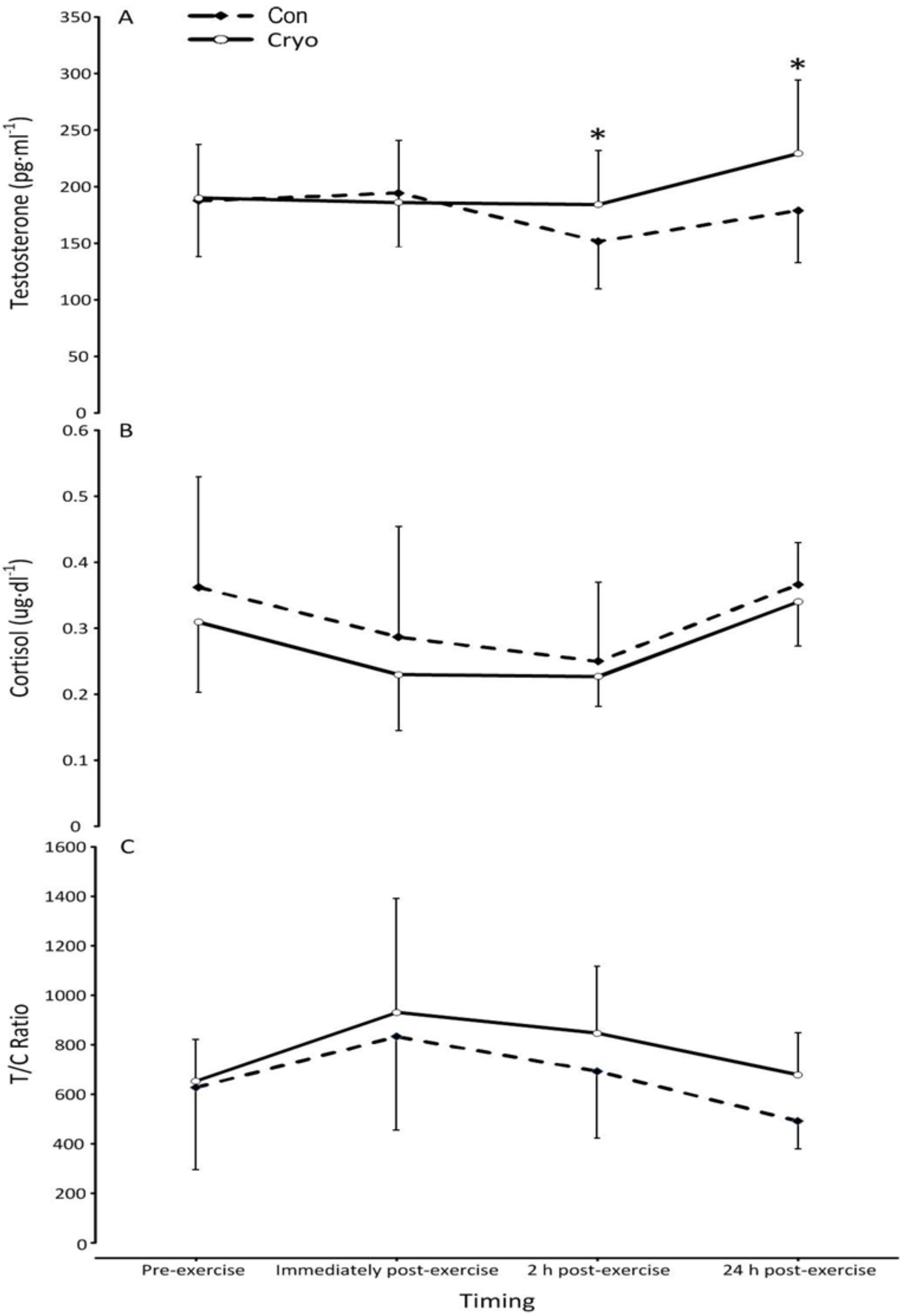

Figure 1: Mean ± SD testosterone (panel A), cortisol (panel B) and testosterone/cortisol ratio (panel C) responses throughout each trial. Con represents control trial, Cryo represents cryotherapy trial. * represents significant difference (P<0.05) between conditions at the corresponding time-point.

TABLES

Table 1: Mean ± SD blood lactate, peak power output, Creatine Kinase, perceived soreness and perceived recovery responses throughout each trial.

Variable	Trial	Timing: Pre-exercise (A)	Timing: Immediately post-exercise (B)	Timing: 2 h post-exercise (C)	Timing: 24 h post-exercise (D)	Significant differences relative to pre-exercise (A)	95% confidence interval for *post hoc* difference
Blood lactate ($mmol \cdot L^{-1}$)	Con	1.21 ± 0.40	3.49 ± 1.29	1.06 ± 0.31	1.29 ± 0.46	A vs. B	1.35 – 3.02
	Cryo	1.06 ± 0.39	3.15 ± 1.14	1.22 ± 0.38	1.33 ± 0.36		
Peak power output (W)	Con	4151 ± 494	4004 ± 443	4055 ± 489	4089 ± 459	A vs. B	-216 – -51
	Cryo	4092 ± 466	3971 ± 482	4009 ± 406	4127 ± 468		
Creatine Kinase ($\mu \cdot L^{-1}$)	Con	232 ± 44	261 ± 53	291 ± 59	785 ± 129	A vs. B	8 – 57
	Cryo	232 ± 49	269 ± 63	303 ± 65	799 ± 141	A vs. C	46 – 83
						A vs. D	452 – 668
Perceived soreness (units)	Con	1 ± 1	3 ± 2	2 ± 1	2 ± 2	A vs. B	1 – 3
	Cryo	1 ± 1	3 ± 2	1 ± 1	2 ± 2		
Perceived recovery (units)	Con	6 ± 2	3 ± 2	6 ± 2	6 ± 2	A vs. B	-4 – -1
	Cryo	7 ± 2	4 ± 2	7 ± 2	6 ± 3		

Con represents control trial, Cryo represents cryotherapy trial.